

Supporting Information

Injectable Gelatin Microgel-based Composite Ink for 3D Bioprinting in Air

Kaidong Song^{1, §}, Ashley M. Compaan^{2, §, #}, Wenxuan Chai¹, Yong Huang^{1, 2, *}

[§] Equal contribution

¹Department of Mechanical and Aerospace Engineering, University of Florida, Gainesville, FL 32611, USA.

²Department of Materials Science and Engineering, University of Florida, Gainesville, FL 32611, USA.

[§] Equal contribution

[#]Novabone Products, LLC. 13510 NW US Highway 441, Alachua, FL, 32615 USA.

^{*}Corresponding author, yongh@ufl.edu

Supporting Information SI1

Rheological property measurement of gelatin composite ink.

In this study 3% gelatin solutions with 5%, 10%, and 15% gelatin microgels were used here for rheology property measurements accordingly. The yield-stress property was investigated by sweeping the shear stress and recording the shear moduli and is shown in **Figure S1a**. The yield stress is a value at which a material starts to deform. From **Figure S1a**, it is found that with the increase of the gelatin concentration, the yield stress increases.

The shear-thinning behavior of the gelatin microgel-based composite ink with different concentrations was measured, and the corresponding viscosity changes are shown in **Figure S1b**. The viscosity of the gelatin microgel suspensions with different concentrations decreases with the increase of shear rate. This shear-thinning behavior is considered as a result of rearrangements of microscale structures within the fluid during shearing. **Figure S1b** also illustrates that the viscosity increases with the gelatin concentration. Since the viscosity is the property of a fluid-like material to resist deformation, a higher concentration of gelatin causes a higher resistance to deformation.

The reversible jamming-unjamming transition was characterized by shearing and resting for several cycles, and the shear moduli changes are shown in **Figure S1c** and **S1d**. It is found that in the resting condition, the gelatin microgels in the suspension jam together to present solid-like behavior, while under shear the unjammed microgel particles enable the suspension to behave like a liquid. All the suspensions at different gelatin concentrations and temperatures can reversibly switch the states between fluid- and solid-like in a relatively short time.

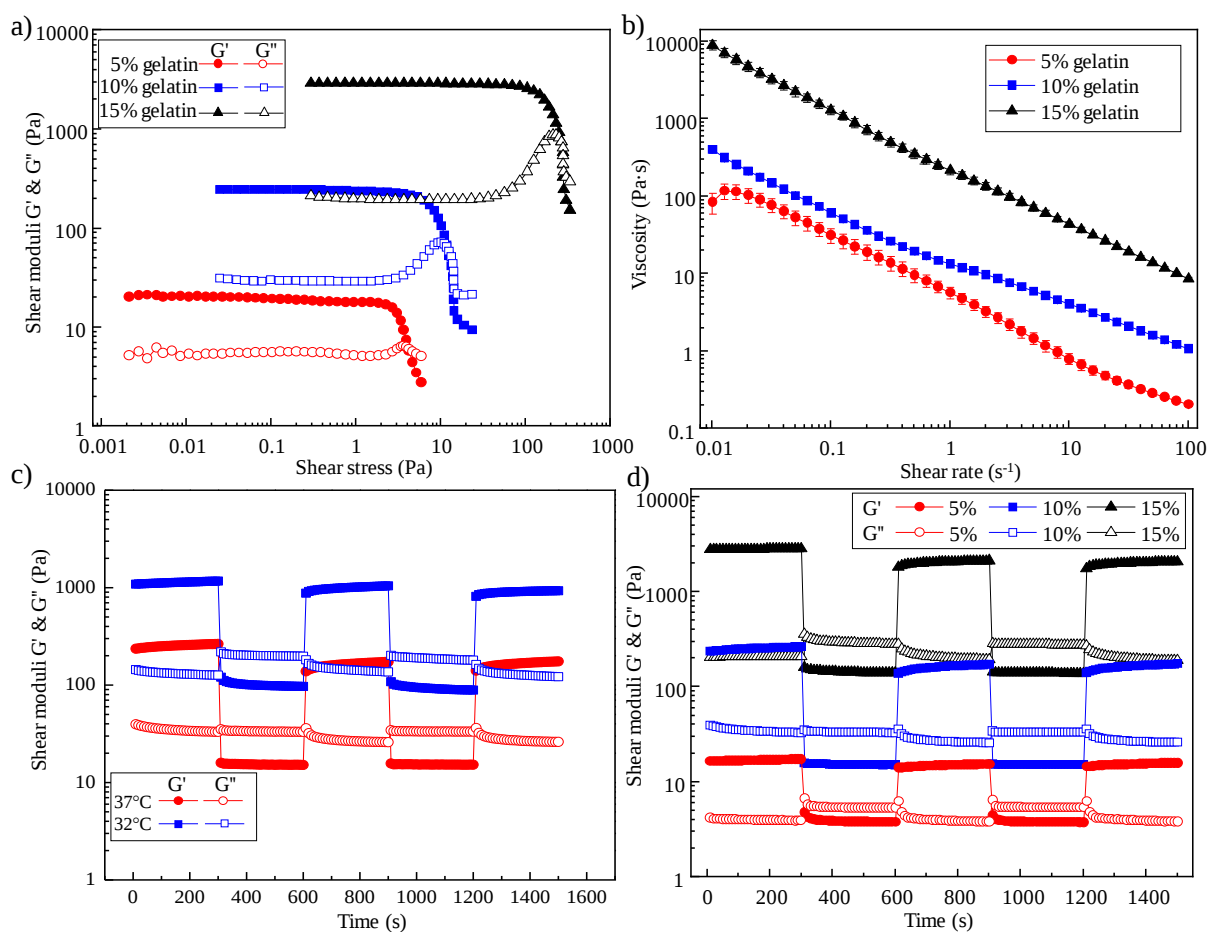


Figure S1. Rheological property measurements. (a) Shear modulus as a function of shear stress of gelatin microgel-based composite ink formulations at 37°C. (b) Viscosity as a function of the shear rate to illustrate the shear-thinning behavior. (c) Shear-thinning and recovery through low and high strain cycles at different temperatures when using 10% gelatin microgels. (d) Shear-thinning and recovery through low and high strain cycles with different microgel gelatin concentrations.

Supporting Information SI2

Effect of temperature on composite ink extrusion.

The temperature effects on the composite ink printability were studied as shown in **Figure S2a**, **S2b** and **S2c** when using a 3% gelatin with 10% gelatin microgels, and no TG composite ink. The printing quality at a lower temperature (**Figure S2a**) (below 30°C) is worse than that when printing above the critical point (upper critical sol-gel transition temperature (UCST)) (**Figure S2b**). Although the general shape of the steady shear rheology curves shown in **Figure 2a** is similar, it shifts toward higher shear stresses and higher apparent viscosities as the temperature decreases, which indicates stronger solid-like behavior and more resistance for the composite ink(s) to flow. During material extrusion and printing, this means intermittent nozzle clogging and poor printing performance. **Figure S2c** shows that the lines printed at a higher temperature have higher quality although the low yield stress may limit the achievable height and complexity of printed constructs at this temperature.

The microgel effects on the composite ink printability were compared as presented in **Figure S2b** and **S2d**. Since the microgels can impart yield-stress behavior in which the extruded composite ink can recover to the solid-like state quickly, the corners of the printed spiral pattern with the microgels are sharper than those without the microgels.

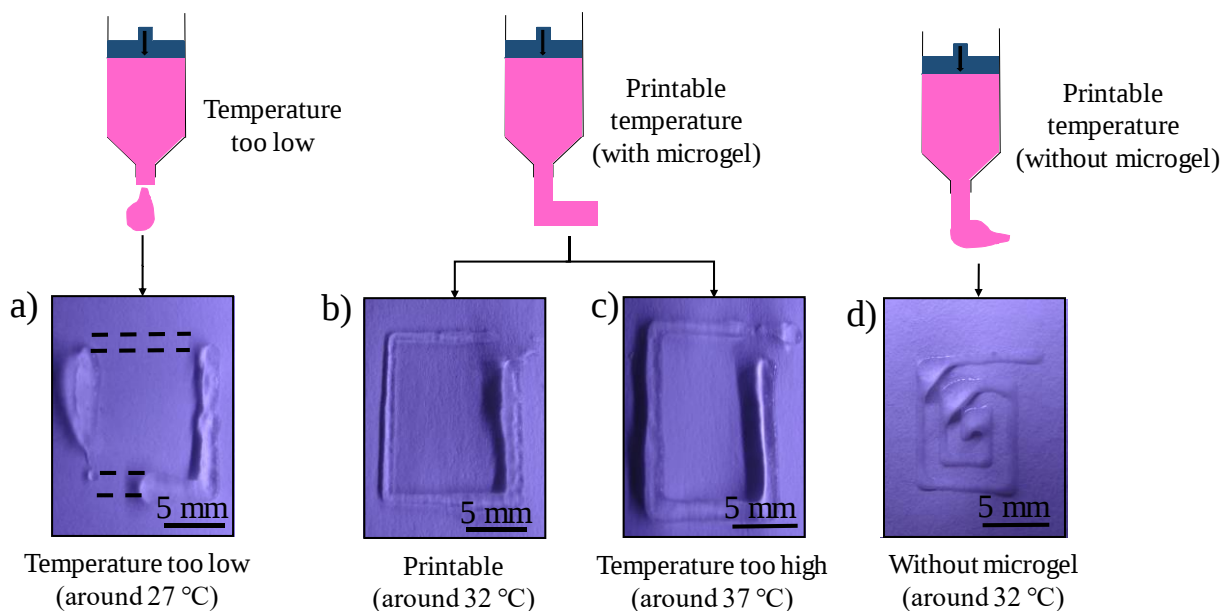


Figure S2. Effects of temperature and microgel on the composite ink printability. Filament extruded at a low temperature (a), working temperature (b), and high temperature (c) of the microgel composite ink. Filament morphology under working temperature using the ink without gelatin microgels (d).

The self-supporting capability of the composite ink was studied by calculating the maximum shear stress τ_{max} and the maximum tensile stress σ_{max} of overhang filaments. The filament printed in **Figure 3a** between two posts can be simplified as a uniformly distributed loaded simply supported

beam with a weight-induced load. The maximum shear stress that always occurs at the two end points could be calculated by $\tau_{\max} = \frac{F_s S_{zm \text{ ax}}}{I_z b} = \frac{4F_s}{3A}$, where F_s is the maximum shear force and A is the cross-sectional area of the filament. The maximum force occurring at the two endpoints is: $F_s = \frac{1}{2}qL$, where q is the weight distribution along the beam as $q = \pi\rho gR^2$, where ρ is the density, g is the gravitational acceleration, R is the radius of the beam, and L is the length of the beam. As such, the maximum shear stress could be calculated as $\tau_{\max} = \frac{2}{3}\rho gL$. The maximum tensile stress that always occurs in the midspan can be calculated using $\sigma_{\max} = \frac{M_{\max} y}{I}$, where $M_{\max}$ is the maximum bending moment, y is the distance between the neutral axis and the analyzed surface, which equal to the radius of the filament (R) herein, and I is the moment of inertia. The maximum bending moment is: $M_{\max} = \frac{1}{8}qL^2$. The moment of inertia is: $I = \frac{1}{4}\pi R^4$, and the maximum tensile stress can be rewritten as: $\sigma_{\max} = \frac{\rho g L^2}{2R}$. To determine whether an overhang filament can be printed between two posts, the calculated maximum shear stress and maximum tensile stress are compared with the shear stress τ_{ink} and tensile stress $\sigma_{\max}$ of the designed gelatin microgel-based composite ink. Based on the shear stress of the composite ink at 32°C, which is measured as 301.5 Pa as seen from **Figure 2a**, the tensile stress of the composite ink can be estimated by $\sigma_{ink} = \frac{3}{\sqrt{2}}\tau_{ink}$ according to the octahedral shear stress theory. This results in a tensile stress value of 639.58 Pa. The $\tau_{\max}$ and $\sigma_{\max}$ of the overhang beam are estimated as 65.5 Pa and 610.6 Pa, respectively (based on $L = 10.0$ mm and $R = 0.8$ mm), which are lower than the values of τ_{ink} and σ_{ink} , so the filament does not break up during deposition.

Supporting Information SI3

Mechanical property measurement of gelatin composite parts.

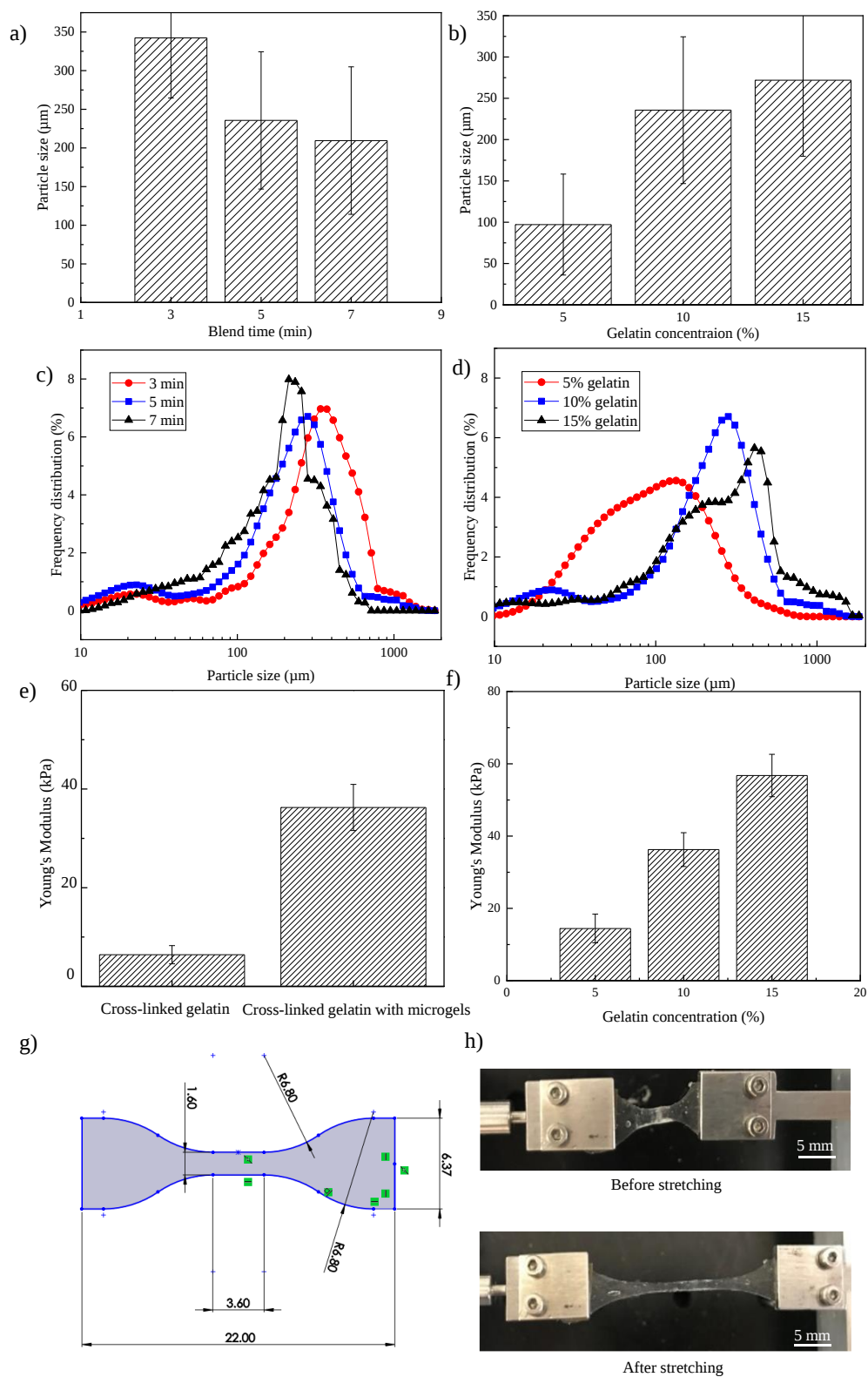


Figure S3. Particle size and mechanical characterization of gelatin composites. Gelatin microgel particle size as a function of (a) blend time and (b) microgel gelatin concentration. Gelatin microgel particle size-frequency distribution as a function of (c) blend time and (d) microgel gelatin concentration. (e) Young's modulus of cast parts using 3% gelatin solution with and without 10% gelatin microgels. (f) Young's modulus of cast parts using 3% gelatin solution with different microgel gelatin concentrations. (g) Dimensions of dog bone-shaped specimens. (h) Gelatin-based specimen (10% gelatin microgels) without elongation and with 20 mm elongation.

To assess the relationship of gelatin microgel size with the blend time and microgel gelatin concentration, the average gelatin microgel size and size-frequency distribution were measured under different blend times and microgel gelatin concentrations. From **Figure S3a** to **S3d**, it is found that the average microgel diameter increases with the increase of the gelatin concentration using the same blend time. In contrast, the microgel size decreases with the increasing blend time and approaches a plateau after 5 min. Therefore, 5 min has been chosen herein for blending.

To evaluate the mechanical property of the microgel composites, the Young's modulus of cross-linked gelatin with (5%, 10%, and 15% gelatin microgels) and without the gelatin microgels were measured. It is found that the addition of the gelatin microgels (10%) to the gelatin solution (3%) increases the Young's modulus as illustrated in **Figure S3e**, where the Young's modulus of the cross-linked 3% gelatin is 6.4 kPa without adding any gelatin microgels and is 36.3 kPa with the addition of the 10% gelatin microgels. With the increase of microgel gelatin concentration, the Young's modulus increases as shown in **Figure S3f**; the Young's moduli are approximately 14.4 kPa, 36.3 kPa, and 56.8 kPa, respectively. For illustration, the schematic of the designed dog bone-shaped structure is shown in **Figure S3g**, and the specimen at rest and under tension (20 mm extension) is shown in **Figure S3h**.

Supporting Information SI4

Analysis of the dimension fidelity of printed structures.

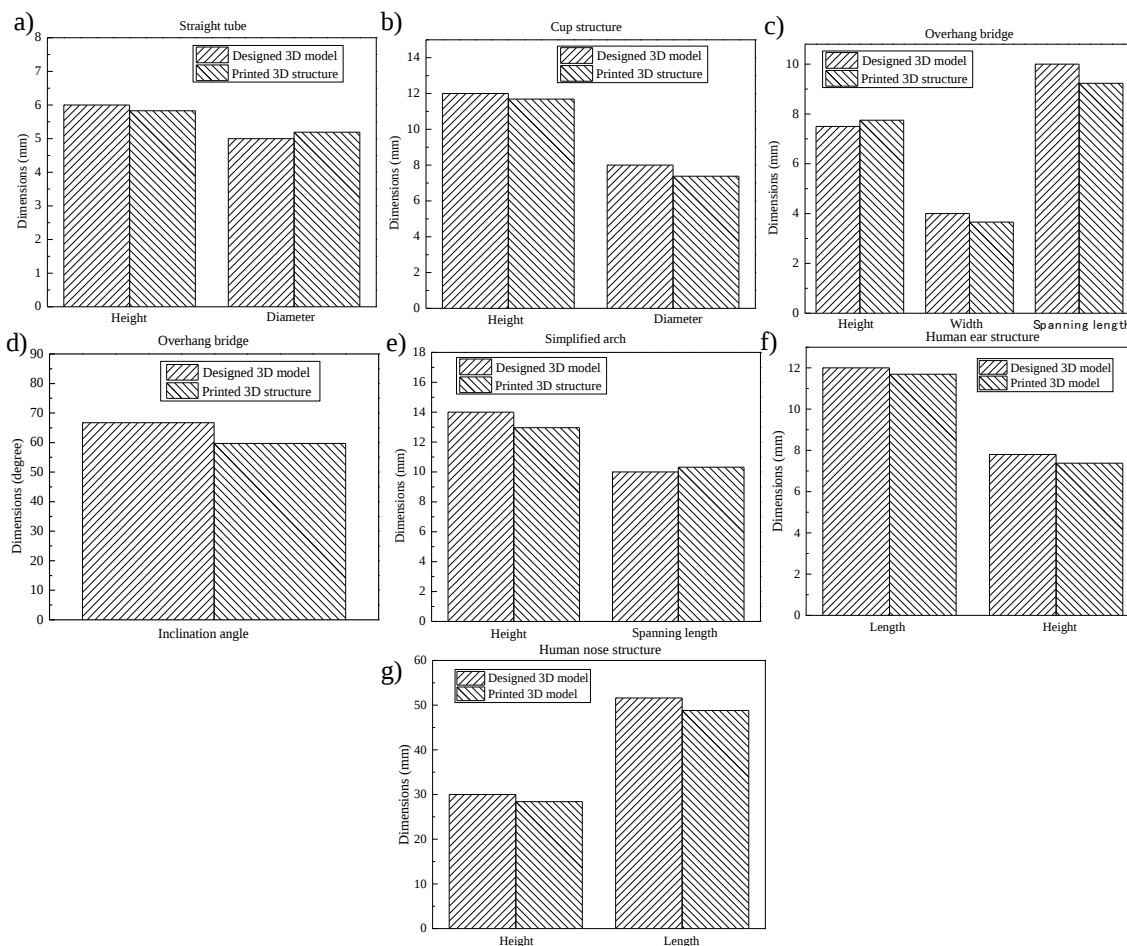


Figure S4. Comparison of dimensions between designed 3D models and printed 3D structures for (a) straight tube, (b) cup structure, (c) and (d) overhang bridge, (e) simplified arch, (f) human ear structure and (g) human nose structure.

To assess the print fidelity of the designed gelatin microgel-based composite ink, the geometries of the printed complex 3D structures: straight tube, cup structure, overhang bridge, simplified triumphal arch, human ear structure, and human nose structure were measured and compared with the designed 3D models. The overall dimensions (as applicable) including the diameter, height, inclination angle, length, and width of the designed 3D models and the printed 3D structure are compared accordingly and shown in **Figure S4**. Slight dimensional differences are attributed primarily to the soft and easily deformable nature of the printed structures. No significant discrepancy is observed between the designed models and the printed 3D structure, which confirms that the gelatin microgel-based composite ink can effectively hold complex structures as a self-supporting material.

Supporting Information SI5

Analysis of water content and volume shrinkage of gelatin composites.

To assess the value of water content in the gelatin composites with different microgel gelatin concentrations (5%, 10% and 15% gelatin microgels), **Figure S5a.** shows the water content as a function of microgel gelatin concentration. Based on the residual weight after drying at 37°C for 24 hours, the water contents were calculated as 11.66 g/g for the 5% gelatin microgel composites, 6.96 g/g for the 10% gelatin microgel composites, and 6.23 g/g for the 15% gelatin microgel composites. All of the designed gelatin microgel-based composite inks have a high water content.

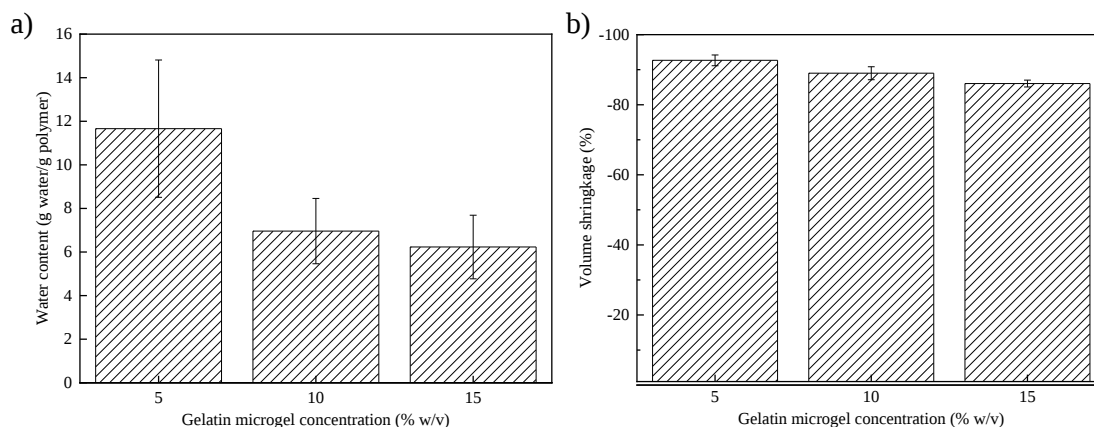


Figure S5. Water content (a) and volume shrinkage (b) of the composite ink with different gelatin concentrations for microgels.

To investigate the structural stability of the designed gelatin microgel-based composite inks, the volume shrinkage of various hydrogel composites after gel formation was compared after a 24-hours drying process. As shown in **Figure S5b**, the volume shrinkage for the 5%, 10%, and 15% gelatin microgel-based composite inks is -92.67%, -88.99%, and -86.05%, respectively. The more concentrated gelatin microgels can decrease the degree of volume shrinkage, which confirms that the addition of the gelatin microgels can improve the volume stability.